<Division of Environmental Chemistry>Molecular Microbial Science

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– Molecular Microbial Science –

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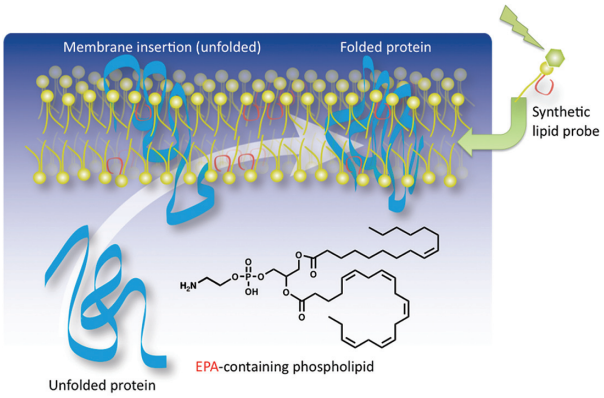
Prof BUCKEL, Wolfgang Max Planck Gesellschaft, Germany, 18 October–14 November

Scope of Research

Microorganisms are found almost anywhere on Earth. They have a great diversity of capacities to adapt to various environments including chemically and physically unusual environments. Our main subject is to clarify the molecular basis of environmental adaptation of microorganisms and their application. Specific functions of proteins and lipids that play essential roles in environmental adaptation of extremophilic microorganisms are of our particular interest. Mechanistic analysis of microbial enzymes, in particular those involved in unique metabolic pathways, and their application are also undertaken.



KEYWORDS

Molecular Microbial Science
Biochemistry
Psychrotroph
Polyunsaturated Fatty Acid
Bioengineering

Selected Publications

Kawamoto, J.; Sato, T.; Nakasone, K.; Kato, C.; Mihara, H.; Esaki, N.; Kurihara, T., Favourable Effects of Eicosapentaenoic Acid on the Late Step of the Cell Division in a Piezophilic Bacterium, *Shewanella violacea DSS12, at High-Hydrostatic Pressures, Environ. Microbiol.*, **13,** 2293-2298 (2011).

Sato, S. B.; Sato, S.; Kawamoto, J.; Kurihara, T., Differential Roles of Internal and Terminal Double Bonds in Docosahexaenoic Acid: Comparative Study of Cytotoxicity of Polyunsaturated Fatty Acids to HT-29 Human Colorectal Tumor Cell Line, *Prostaglandins Leukot. Essent. Fatty Acids*, **84,** 31-37 (2011).

Usui, K.; Hiraki, T.; Kawamoto, J.; Kurihara, T.; Nogi, Y.; Kato, C.; Abe, F., Eicosapentaenoic Acid Plays a Role in Stabilizing Dynamic Membrane Structure in the Deep-Sea Piezophile *Shewanella violacea*: A Study Employing High-Pressure Time-Resolved Fluorescence Anisotropy Measurement, *Biochim. Biophys. Acta*, (2011) (in press).

Vasudevan, A.; Fujita, M.; Kurata, A.; Kawamoto, J.; Esaki, N.; Kurihara, T., Function of $FADH_2$Dependent 2-Haloacrylate Hydratase from a 2-Chloroacrylate-Utilizing Bacterium, *Burkholderia sp. WS, Trace Nutrients Research*, **28,** 58-64 (2011).

Hidese, R.; Mihara, H.; Kurihara, T.; Esaki, N., *Escherichia coli* Dihydropyrimidine Dehydrogenase Is a Novel NAD-Dependent Heterotetramer Essential for the Production of 5,6-Dihydrouracil, *J. Bacteriol.*, **193,** 989-993 (2011).

Eicosapentaenoic-Acid-Containing Phospholipids Act as a Chemical Chaperone for the Rapid Folding of a Cold-Inducible-Membrane Protein

A cold-adapted microorganism, *Shewanella livingstonensis* Ac10 isolated from Antarctic seawater, produces eicosapentaenoic acid (EPA) as an acyl chain of its membrane phospholipids at 4°C. When EPA-biosynthesis genes were disrupted, the EPA-lacking mutant showed the growth retardation and formed filamentous cells at 4°C, but not at 18°C, suggesting that EPA-containing phospholipids have an important role in the cold adaptation of this bacterium. We also found that, in the EPA-lacking mutant grown at 4°C, a cold-inducible outer membrane protein, Omp74, forms different conformation from that in the parent strain. In order to elucidate the physiological role of EPA in the folding of Omp74, we performed *in vitro* reconstitution of recombinant Omp74 with the liposomes containing or not containing EPA and analyzed the effect of the presence of EPA on the folding of Omp74. The larger amounts of folded Omp74 were observed in the liposomes containing EPA than those without EPA. Circular dichroism analysis indicated that Omp74 rapidly interacts with the membrane surface and rearranges its β-sheet structures in the EPA-containing liposome at 4°C. These results suggest that EPA-containing phospholipids accelerate the secondary structure formation and folding of Omp74 and play a role as a molecular chaperone of Omp74 at low temperatures.

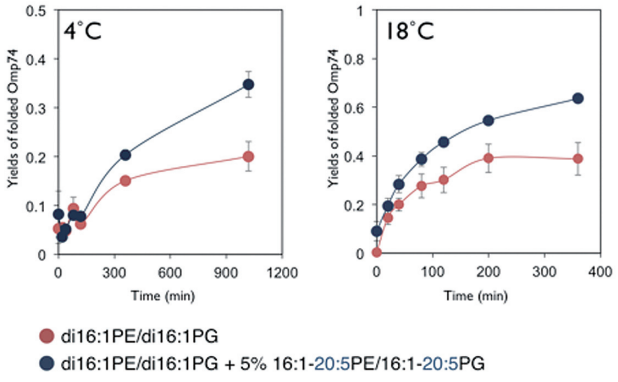
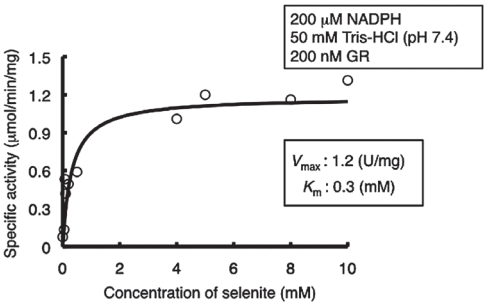


Figure 1. EPA-containing phospholipids induce the folding of a cold-inducible porin, Omp74, at low temperatures.

Studies on the Transport and Metabolism of Selenium in Mammalian Cells

Selenide is a precursor of selenophosphate, which is the active selenium donor compound required by bacteria and mammals for the synthesis of selenocysteine in selenoproteins. Although selenite is the main source for selenide, selenite reduction pathway is still largely unclear. It has been suggested that selenite is reduced by glutathione (GSH) and thioredoxin reductase (TrxR). However, these pathways have not been demonstrated in living cells. Especially, selenoprotein biosynthesis was not affected by knock down of TrxR. In this study, we evaluated the physiological role of GSH in selenoprotein biosynthesis by using hepa1-6 cells. When we used buthionine sulphoximine (BSO), a selective GSH depleting agent, decrease in the production of selenoproteins was not observed. On the other hand, under the same conditions, the amounts of intracellular selenium compounds were increased. These results revealed the presence of unknown pathway for the intracellular selenium reduction. In order to explore a new mechanism for selenite reduction, we focused on glutathione reductase (GR), a homolog of TrxR, and analyzed its selenite-reducing activity *in vitro*, demonstrating that hydrogen selenide is generated as NADPH decreases under anaerobic conditions. This finding raises the possibility that GR is involved in the *in vivo* selenite reduction pathway.



$$H_2SeO_3 + 3NADPH + 3H^+ \xrightarrow{GR} H_2Se + 3NADP^+ + 3H_2O$$

Figure 2. Selenite reducing activity of glutathione reductase (GR).